FISH TAGGING WITH INJECTED DYES

P.A. RYAN

Department of Zoology, University of Canterbury,
Christchurch, New Zealand

ABSTRACT

The literature on tagging fish with injected dyes is briefly reviewed and the result of experimentally marking the New Zealand short-finned eel *Anguilla australis schmidtii* with a jet inoculator and a fluorescent tag is reported.

INTRODUCTION

It is often necessary to tag fish when trying to obtain data on fish populations. An ideal marking method should make any fish permanently and unmistakably recognisable (preferably individually) and yet have no effect on its growth, mortality, or behaviour, or increase its liability to capture by a predator or by fishing gear (Stott 1968). Unfortunately with present techniques it is impossible to reach these standards although non-mechanical tags, such as coldbranding, immersion staining, biological marks, tattooing and subcutaneous injection go a long way towards reaching these criteria. Of these tags, tattooing and subcutaneous injection appear to offer the most promise.

REVIEW OF MARKING METHODS

Stains and dyes have been used by several workers in the last two decades for marking fish in population and growth studies. These are summarised in Table 1. Jackson (1959) pioneered a technique for mass marking small fish. This utilised a sandblasting gun to subepidermally tag fingerlings with biologically inert fluorescent polystyrene. Fish were then viewed under ultraviolet light to observe the fluorescing stain. Phinney *et al.* (1967) experimented with different particle sizes of polystyrene and air pressures in tagging pink salmon, *Oncorhynchus gorbuscha*, coho salmon, *O. kisutch*, sockeye salmon, *O. nerka* and rainbow trout, *Salmo gairdneri*. They found between 28.3 and 100% retention of the marks after 130 days. Duncan and Donaldson (1968) tested mark retention time on salmonid fingerlings tattooed with fluorescent pigment and found clear marks remained for over two years. Phinney and Mathews (1969) studied the effects of spray tagging age 0 coho salmon by comparing their growth and survival with fin-clipped and control fish over a period of six months. No differences were found between control and pigment marked fish, but fin-clipped fish suffered higher mortalities and lower growth rates. Phinney and Mathews (1973) followed up their earlier work on coho salmon

by testing long term retention times of spray marked fingerlings. They found no statistically significant loss of pigment over a two year test period. Hennick and Tyler (1970) marked pink salmon fry by spraying with fluorescent pigment and found fish mortalities increased with an increase in spraying pressure. Retention rate was not dependent on pressure so the authors concluded that the lowest effective spraying pressure, 70 psi (482 kPa), was most satisfactory as there was no mortality at this pressure.

Andrews (1972) pointed out that no experiments on mark longevity of fluorescent pigment or related marking mortalities had been done on cyprinids. He therefore carried out experiments on the fathead minnow, *Pimephales promelas* applying pigments with the sandblasting technique of Jackson (1959). Three colours, red, green, and yellow were tested. Of these red and yellow gave the best visibility. All fish retained marks until the end of the study (232 days) but the best retention was on the operculum and the bases of the pectoral and pelvic fin rays.

Rinne and Deacon (1973) attempted to mark fish by immersion staining and by spraying with fluorescent pigments. Three dyes were used for immersion staining: Trypan Blue, Bismarck Brown Y and Rhodamine B, but on one of the species tested, *Lepidomeda mollispinis*, all were rejected because of poor dye uptake and severe stress. The other species, *Cyprinodon nevadensis*, was stained only by Bismarck Brown Y and retention time was for three to four days only. Fluorescent pigment spraying produced a mark which was visible under ultraviolet light and which remained during the experimental period, or if less, for the life of the fish.

Hypodermically injected dyes were used successfully by Bond and Culver (1952) on cutthroat trout *Salmo clarkii clarkii*. Fish were injected with Trypan blue, producing marks which lasted for three weeks. Al-Hamid (1954) tried a number of dyes and found several that produced effective marks on the bluegill, *Lepomis macrochirus*, for up to 45 days. Hansen and Stauffer (1964) used mercuric sulphide and cadmium sulphide to mark sea lamprey larvae, *Petromyzon marinus*, by subepidermal injection in the body. These marks remained for up to four years.

Kelly (1967) used intracutaneous injection to mark brown trout, *Salmo trutta*. He screened over 150 chemicals for this purpose. Of these, National Fast Blue 8 GXM, a water soluble blue dye, and hydrated chromium oxide, an insoluble green pigment, were best. He also tested a Press-O- Jet inoculator, a high pressure jet sprayer, for introducing dyes subepidermally. This appeared to be highly successful, and produced mark retention times of at least 18 months. The inoculator has an advantage over hypodermic syringes in that it can be used to tag fins as well as scaled areas. It is also faster to use, and there is less risk of damaging fish. Hart and Pitcher (1969) also tried this technique on a variety of fish using Alcian Blue, a dye related to National Fast Blue 8 GXM. This dye worked admirably, and gave mark retention times of up to 14 months in laboratory trials and 11 months in field trials. Smith (1970) hypodermically injected the sticklebacks, *Culaea inconstans* and *Pungitius pungitius*, with a fluorescent pigment but this technique required the use of an anaesthetic to immobilise the

fish and, in the case of small fish, a dissecting microscope. As Smith pointed out this would tend to limit the usefulness of the technique when large numbers of fish are being marked. Even so the method provides individually marked fish which retain their marks for over eight months.

Finally acrylic colours appear to offer promise for individually marking fish subcutaneously as at least nine readily differentiable colours are available. Lotrich and Meredith (1974) injected an acrylic polymer emulsion into the base of the caudal peduncle of a number of different fish species in field trials and the colours were retained for at least four months. The authors concluded that the technique provides an economical and rapid method for marking large numbers of small fish.

USE OF DYES IN NEW ZEALAND FISH STUDIES

New Zealand workers have been slow to use dye marking methods and most of the literature on the subject is unpublished. Cadwallader (1973) used Alcian Blue to finmark *Galaxias vulgaris* with a jet inoculator similar to the one used by Hart and Pitcher (1969). He anaesthetised the fish, laid them flat on a wet cloth and administered the dye with the nozzle of the inoculator 2-3 mm from the marking site. Cadwallader found mark retention times of over 11 months both in the laboratory and in the field. Pontamine Fast Pink was also tried in laboratory situations but marks made with this dye faded after three weeks. I have used the same equipment to differentially finmark migrant short finned eels, *Anguilla australis schmidtii*, in laboratory experiments. Marks remained visible for three months but some difficulty was found in differentiating the marks from the dark fin colour.

PRELIMINARY MARKING STUDY USING A JET INOCULATOR AND A FLUORESCENT PIGMENT

Surprisingly, no one has reported the use of both a jet inoculator and fluorescent pigment which seem a logical combination. Fluorescent pigments are preferable to the visible substances used by Kelly (1967) because they are usually invisible in ordinary daylight. This reduces the chance of possible selective mortality of fish associated with a brightly visible mark. The jet inoculating technique used by Kelly (1967), Hart and Pitcher (1969) and Cadwallader (1973) is preferable to the sandblast-gun spraying technique because it reduces mortality and it allows differential marking. Sandblast spraying is most suitable for mass marking fish.

METHODS

To test retention times of marks produced by jet inoculation in conjunction with fluorescent pigments, a small experiment was set up in October 1973. Ten non-migrant short finned eels ranging in length from 40-60 cm were fyke netted in Lake Ellesmere, Canterbury (172°30'E, 43°45'S) and stored in concrete containers. A suspension of Sterling Yellow fluorescent pigment S.100* in saline was prepared and inoculated with a jet

* Sterling Colour Company Ltd, Sterling House, Heddon Street, London, W.1.

TABLE 1. SUMMARY OF DYES, MARKING METHODS AND MARK RETENTION TIMES FOR DIFFERENT SPECIES OF FISH.
- = Retention time not known. American vernacular fish names according to Carlander (1969).

Authority	Species	Dye	Method	Retention time lab.	Retention time field
Bond and Culver 1952	*Salmo clarkii clarkii* (Cut throat trout)	Trypan Blue	Hypodermic	-	3 weeks
Al-Hamid 1954	*Lepomis macrochirus* (Bluegill)	Brilliant Vital Red	"	40 days	-
		Alizarin Red S		40 "	-
		Congo Red		40 "	
		Chlorazol Fast Pink B		40 "	-
		Nigrosine		70 "	-
		Trypan Blue		36 "	
		Fast Green B		50 "	-
		Indigo Carmine		5 "	-
		Trypan Red		Killed fish	
		Safranin O		"	
		Gentian Violet		"	
Jackson 1959	*Lepomis* sp. (Common sunfish)	Derbylite	Sandblasting	-	-
	Salmo gairdneri (Rainbow trout)	"	"	-	-
	Perca flavescens (Yellow perch)	"	"	-	-
	Salvelinus fontinalis (Brook trout)	"	"	-	-
	Salvelinus namaycush (Lake trout)	"	"	-	-
	Esox sp. (Pickerel)	"	"	-	-
	Ictalurus sp. (Hornpout)	"	"	-	-
	Fundulus sp. (Killifish)	"	"	-	-
Hansen and Stauffer 1964	*Petromyzon marinus* (Sea lamprey)	Mercuric sulphide	Hypodermic	-	4 years
		Cadium sulphide		-	4 "
Kelly 1967	*Salmo trutta* (Brown trout)	National Fast Blue 8 GXM	Hypodermic	24 months	-
		hydrated chromium oxide		12 "	-
	Salvelinus fontinalis	"	"	12 "	12 months
				12 "	12 "
	Pseudopleuronectes americanus (Winter flounder)	"	"	>4 "	-
				>4 "	-
	Micropterus dolomieui (Smallmouth bass)	hydrated chromium oxide	"	>6 "	-
	Notemigonous crysoleucas (Golden shiner)	National Fast Blue 8 GXM	"	>6 "	-
	Perca flavescens	"	"	>6 "	-
	Lepomis gibbosus (Pumpkinseed)	"	"	>6 "	-
	Cyprinus carpio (Carp)	"	"	>6 "	
Duncan and Donaldson 1968	*Oncorhynchus kisutch* (Coho salmon)	Day Glo Pigment	Tattoo	-	29 months
Pitcher and Hart 1969	*Rutilus rutilus* (Roach)	Alcian Blue	Jet inoculator	4 months	-
	Squalius cephalus (Chub)	"	"	14 "	11 months
	Leuciscus leuciscus (Dace)	"	"	14 "	11 "
	Alburnus alburnus (Bleak)	"	"	-	5 "
	Barbus barbus (Barbel)	"	"	-	10 "
	Gobio gobio (Gudgeon)	"	"	-	10 "
	Phoxinus phoxinus (Minnow)	"	"	14 months	11 "
	Perca fluviatilis (Perch)	"	"	-	4 "
	Acerina cernua (Ruffe)	"	"	-	3 "
	Esox lucius (Pike)	"	"	-	3 "

(Table 1 continued)

Authority	Species	Dye	Method	Retention time lab.	Retention time field
Pitcher and Hart 1969 (Continued)	*Noemacheilus barbatula* (Stone loach)	Alcian Blue	Jet inoculator	-	3 months
	Cottus gobio (Bullhead)	"	"	-	8 "
Smith 1970	*Culaea inconstans* (Brook stickleback)	Tracer-Glo	Tuberculin syringe	-	>8 "
	Pungitius pungitius (Nine spine stickleback)	"	"	-	>11 "
	Pimephales promelas (Fathead minnow)	"	"	-	>8 "
Andrews 1972	*Pimephales promelas*	Granular fluorescent pigment	Sandblasting gun	-	>20 "
Ryan 1972	*Anguilla australis schmidtii* (Short-finned eel)	Alcian Blue	Jet inoculator	-	>3 "
Cadwallader 1973	*Galaxias vulgaris*	"	"	>11 months	11 months
Phinney and Mathews 1973	*Oncorhynchus kisutch*	Granular fluorescent pigment	Sandblasting gun	-	>2 years
Rinne and Deacon 1973	*Lepidomeda mollispinis* (Virgin River Spinedace)	"	"	-	>85 days
	Cyprinodon nevadensis (Nevada pupfish)	"	"	-	>10 months
Lotrich and Meredith 1974	*Etheostoma flabellare*	Acrylic polymer emulsions	Tuberculin syringe	-	2-16 "
	Etheostoma caeruleum	"	"	-	2-16 "
	Etheostoma nigrum	"	"	-	2-16 "
	Etheostoma sagitta	"	"	-	2-16 "
	Ericymba buccata (Silverjaw minnow)	"	"	-	2-16 "
	Semotilus atromaculatus (Creek chub)	"	"	-	2-16 "
	Notropis chrysocephalus (Common shiner)	"	"	-	2-16 "
	Campostoma anomalum (Stone roller)	"	"	-	2-16 "
	Catostomus commersoni (White sucker)	"	"	-	2-16 "
	Fundulus heteroclitus (Zebra or Common killie)	"	"	-	2-16 "
Ryan 1974	*Anguilla australis schmidtii*	Granular fluorescent pigment (yellow)	Jet inoculator	16 months	-

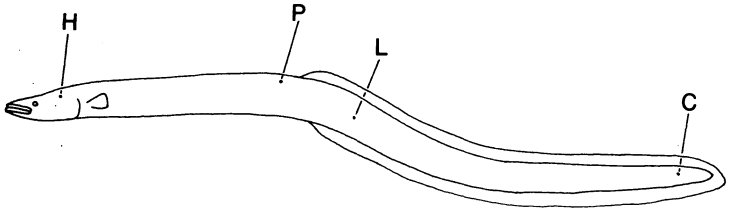

Fig. 1. Tag positions on eels. H, head; P, predorsal; L, lateral; C, caudal.

inoculator from a distance of 2-3 mm. Eels were not anaesthetised but hand-held on a V shaped measuring board. Fish were tagged in four positions on the body (Fig. 1). To examine the tags, eels were viewed under ultraviolet light while swimming at the surface of the water.

RESULTS AND DISCUSSION

Tag retention was good except in the caudal region, and in all ten fish the head tag was still visible after 16 months (Table 2). In nine fish, predorsal and lateral tags were also visible throughout this time, but only two caudal tags lasted longer than three months. Poor retention time in the caudal region may be due to difficulty in satisfactorily inoculating the flailing tail rather than to the unsuitability of the site.

TABLE 2. VISIBILITY OF MARKS ON TEN EELS AFTER THREE TIME INTERVALS

Region inoculated	Number visible		
	1 month	6 months	16 months
Head	10	10	10
Pre-dorsal	10	10	9
Lateral	10	10	9
Caudal	3	2	0

The results given in Table 2 indicate that jet inoculation used in conjunction with fluorescent pigments fulfils many of the criteria of a perfect tag. Using a combination of colours any fish can be differentially marked. The bigger the fish, however, the greater the number of possibilities, as the increased surface area allows more precise positioning of tags. The sand-blasting method of Jackson (1959) was successfully used by him on echinoderms and amphibians. It also seems likely that the jet inoculator would work well on these and perhaps other groups of animals. Research into the use of jet inoculation and fluorescent tags on other classes could prove rewarding.

ACKNOWLEDGMENTS

I wish to thank the University of Canterbury library staff for their friendly service and help in obtaining literature and Dr M. Winterbourn for criticism of the manuscript.

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